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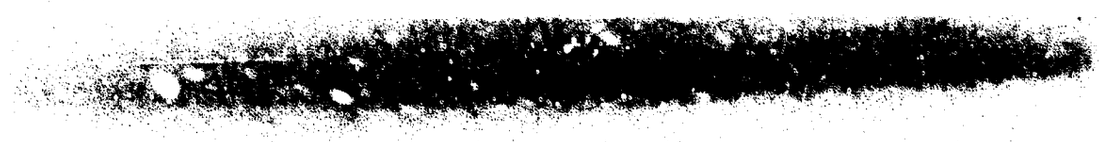

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TECHNICAL MANUSCRIPT 230

NATURAL RESISTANCE OF HAMSTER CELLS TO 8-AZAGUANINE

Alan Richter

JULY 1965

UNITED STATES ARMY
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U.S. ARMY BIOLOGICAL LABORATORIES
Fort Detrick, Frederick, Maryland

TECHNICAL MANUSCRIPT 230

NATURAL RESISTANCE OF HAMSTER CELLS TO 8-AZAGUANINE

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ABSTRACT

In studies designed to introduce mutations into selected animal cell lines in culture, cell lines derived from the Syrian hamster were found to be naturally resistant to 8-azaguanine. Resistance to the drug was measured by plating colony-forming units at different drug concentrations and determining the concentration at which 50% of the colony-forming units formed colonies. By this technique it was shown that KB cells (human) and L cells (mouse) have approximately the same susceptibility to 8-azaguanine as reported by others for L, D98 (human), and P388 (mouse). On the other hand, both P113, a polyoma-transformed derivative of embryonic hamster kidney, and BHK21C13, an untransformed hamster cell displaying contact inhibition, proved 50 times more resistant than the cell lines of human and murine origin. The higher resistance of the hamster cells to 8-azaguanine is specific, since these cells are still susceptible to the unrelated drug 5-bromodeoxyuridine. Preliminary results suggest that the basis for natural resistance is not loss of inosinic-guanylic acid pyrophosphorylase activity, because the cells form colonies under conditions in which growth depends on an exogenous supply of hypoxanthine (HAT medium). The naturally resistant hamster cells therefore resemble the mutant D98/AG described by Szybalski.

NATURAL RESISTANCE OF HAMSTER CELLS TO 8-AZAGUANINE

In our laboratory we have been characterizing several animal cell lines for drug susceptibility preparatory to the selection of specific enzyme-deficient mutants. We have been primarily interested in mutants deficient in inosinic-guanylic acid pyrophosphorylase activity that display resistance to 8-azaguanine as a consequence of enzyme loss, and in mutants deficient in thymidine kinase activity that display resistance to 5-bromodeoxyuridine.

Susceptibility was determined by plating cells in Eagle's medium containing the drug and counting the number of colonies that formed in the presence and absence of the drug. The number of cells forming colonies in the presence of the drug divided by the number of cells forming colonies in the absence of the drug is expressed in per cent as the relative plating efficiency. By plotting graphically the relative plating efficiency at different concentrations of the drug, it is possible to determine the concentration of the drug that gives a relative plating efficiency of 50% or the LD_{50} concentration of the drug. Data for the susceptibility of cell lines to 8-azaguanine were available in the literature for mouse strain L cells, mouse strain P388,[1] and human strain D98.[2] All of these cells were inhibited 50% by drug concentrations between 2×10^{-7} and 4×10^{-7} M 8-azaguanine.

We first tested L cells and KB (human) cells. The data are shown in Tables 1 and 2. Each value represents the average of five dishes. By plotting the data of Tables 1 and 2 graphically, it can be shown that the LD_{50} concentration of 8-azaguanine for L cells is about 2×10^{-7} M and that the LD_{50} concentration for KB cells is about 4×10^{-7} M. Table 3 compares the data for L cells with those published in the literature for P388 and D98. The LD_{50} concentrations are similar.

We next examined a hamster cell called P113, which was isolated by Dr. Defendi* after polyoma infection of embryonic hamster kidney. This cell line was unaffected by concentrations of 8-azaguanine that completely eliminated populations of L or KB cells. By employing increasing concentrations of the drug we found that 2×10^{-6} M 8-azaguanine was necessary to halve the plating efficiency of this cell. To test a possible association between 8-azaguanine resistance and polyoma transformation, we examined a so-called normal hamster cell, BHK21C13.[3] However, the latter proved to be resistant at the same level of drug as the transformed line. This is shown in the fourth table. The increased resistance of the hamster cells to 8-azaguanine is specific, since these cells are still highly susceptible to the unrelated drug 5-bromodeoxyuridine. Preliminary

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* Dr. Vittorio Defendi, Wistar Institute, Philadelphia, Pa.

results suggest that the basis for natural resistance is not the loss of inosinic-guanylic acid pyrophosphorylase activity because the cells form colonies under conditions in which growth depends upon an exogenous supply of hypoxanthine in the medium (hypoxanthine, aminopterin, thymidine). Growth in HAT medium is considered to be an indirect test for the enzyme function. It thus seems that the naturally resistant hamster cells resemble the mutant described by Szybalski[8] as D98/AG, which is resistant to 8-azaguanine but possesses the pyrophosphorylase function. Attempts to select enzyme-deficient mutants from the hamster cells are in progress.

TABLE 1. EFFECT OF 8-AZAGUANINE ON THE RELATIVE PLATING EFFICIENCY OF L CELLS

M Concentration of Drug	Cell Input, 90 per dish			Cell Input, 180 per dish		
	Average No. Colonies Found Per Dish	Plating Efficiency	Relative Plating Efficiency	Average No. Colonies Found Per Dish	Plating Efficiency	Relative Plating Efficiency
0	51.8	57.5	100	88	48.8	100
1×10^{-7}	48.6		94	72.8		82.7
3×10^{-7}	17.4		33.6	24.4		27.7
5×10^{-7}	0.2		0.39	0.2		0.23
8×10^{-7}	0			0		
1×10^{-6}	0			0		

TABLE 2. EFFECT OF 8-AZAGUANINE ON THE RELATIVE PLATING EFFICIENCY OF KB12 CELLS

M Concentration of Drug	Cell Input, 90 per dish			Cell Input, 180 per dish		
	Average No. Colonies Found Per Dish	Plating Efficiency	Relative Plating Efficiency	Average No. Colonies Found Per Dish	Plating Efficiency	Relative Plating Efficiency
0	55.7	61.1	100	119.5	62.2	100
1×10^{-7}	61.4		104	93.0		78.0
3×10^{-7}	42.0		71.6	58.8		49.2
5×10^{-7}	30.4		51.9	5.2		4.4
8×10^{-7}	9.4		16.0	0		(<0.8%)
1×10^{-6}	4.2		7.2	0		(<0.8%)

TABLE 3. SUSCEPTIBILITY OF DIFFERENT CELL LINES TO 8-AZAGUANINE

Drug Concentration	Relative Plating Efficiency, % survival		
	L	P388[a]	D98[b]
1×10^{-7} M	88.4	90	-
3×10^{-7}	30.7	59	-
5×10^{-7}	0.31	10	-
8×10^{-7}	0	0	50
1×10^{-6}	0	0	-

a. Information from Bradley et al.[1]
b. Information from Szybalska and Szybalski.[2]

TABLE 4. DRUG SUSCEPTIBILITY OF CELL STRAINS

Cell	LD_{50} Concentration of Drug[a]	
	8-azaguanine, 10^{-7} molar	5-bromodeoxyuridine, 10^{-6} molar
Mouse strain L	4.5	17.0
Human strain KB	4	8.5
Syrian hamster strain 113	200	2.5
BHK21C13	200	ND[b]

a. Concentration of drug permitting 50% relative plating efficiency compared with plating in absence of drug. All determinations in Eagle's medium containing 10% calf serum.
b. ND - Not determined.

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